Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 6 (2): 153-158, 2011
ISSN 1816-4935 / DOI: 10.3923/jm.2011.153.158

A Study of Antibiotic Susceptibility Pattern of Bacteria Isolates in Sachet Drinking Water Sold in the Cape Coast Metropolis of Ghana

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ABSTRACT

The aim of the study was to determine the antibiotic susceptibility pattern of bacterial isolates in sachet water popularly known as Pure water sold on the street of Cape Coast Metropolis of Ghana. Eleven different sachet water brands were randomly sampled from eleven separate vendors selling those brands of the sachet water in Cape Coast Metropolis of Ghana bi-monthly for six months. A volume of each sample was added to an equal volume of bacteriological peptone water, incubated for 24 h at 37°C and streaked onto Plate Count Agar. All pure isolates were sub-cultured aerobically onto Blood and MacConkey agars for differential purposes. Isolated bacteria were identified morphologically, microscopically and using appropriate biochemical tests. The Kirby-Bauer modified disc diffusion method was used in determining antibiotic sensitivity pattern of bacterial isolates. Bacteria counts ranges between 2.8×10^{5}-5.9×10^{5} cfu mL^{-1} in all Sachet water brands with different bacterial isolates which included *E. coli, Coagulase negative Staphylococcus, S. aureus, E. faecalis, K. aerogenes, M. catarrhalis, B. cereus, L. monocytogenes* and *Enterobacter* sp. etc. The degree of resistance of isolates to the antibiotics ranges from 50.0-87.5%, with multiple drug resistance to 4-7 antibiotics. The isolates showed 100% resistance to Ampicillin, Flucloxacillin and Penicillin, while none of them was resistant to Gentamycin. The resistance to other antibiotics ranged from 93.3% for Erythromycin and Cefuroxime, 60% for Co-trimoxazole and 20% for Tetracycline. The results indicate the presence of antibiotic resistant bacteria in sachet water consumed in the Metropolis with its attendant potential health.

Key words: Bacteria isolate, antibiotics resistance, sachet water, cape coast, ampicillin, gentamycin

INTRODUCTION

Sachet or packaged water is any water that is in a sealed plastic and distributed or offered for sale and is intended for human consumption (Food and Drug Administration, 1995). Water pollution is a major cause of illness (Craun, 1998; Nwachukwu, 2001) and potential health problems may exist due to the microbial content of sachet water since water is one of the vehicles for the transmission of pathogenic organisms (Brock, 1991; Prescott *et al*., 1985). The emergence of bacteria resistant to most of the commonly used antibiotics or drugs is of considerable medical significance (Khan and Malik, 2001) because of the public health implications (Wolday, 1998) reported an increase in incidence of water isolates of multidrug-resistant *Salmonella* in Ethiopia. In Ghana, the supply of piped water is inadequate in most communities. This inadequacy is both in quantity and quality of the public water supply. Only about 10.3 million people (approx. 51%

of the population) are reported to have access to improved water supplies (Gadgil, 1996). This has led to a tremendously increase in the production of sachet water with over 300 registered producers and over 600 unregistered producers in Ghana and according to Food and Drugs Board of Ghana, majority of sachet water are produced under questionable hygienic environmental conditions, without approval and does not meet standards. Regardless of all these problems associated with sachet water, it is considered wholesome for drinking purposes as compared to tap or well water (Addo *et al*., 2009). Research by Dodoo *et al*. (2006), after randomly sampling sachet water in the Cape Coast metropolis between 1999-2004 found them to be bacteriologically very poor, indicating presence of coliforms and other microbes. Research by Kwakye-Nuako *et al*. (2007) found Seventy-seven percent of sachet water sold on the streets of Accra, Ghana to be contained with infective stages of pathogenic parasitic organisms. Thus the objective of this study was to determine bacteria isolates and their antibiotic susceptibility pattern to commonly used antibiotics in sachet water consumed in the Cape Coast Metropolis.

MATERIALS AND METHODS

Materials: Materials, Study site and Period: Samples for the study was obtained from sachet water vendors selling the cold water along some of the streets and markets in the Cape Coast Metropolis. Laboratory analysis was undertaken at the laboratories of the Department of Laboratory Technology and Molecular and Biotechnology of the University of Cape Coast, Ghana. The study was conducted from November, 2009 to March, 2010.

Samples and sampling: The Random sampling technique was used in selecting 11 sachet water brands from 11 different vendors and the brands repeated bi-monthly to ensure different batch numbers were sampled for a period of six months. Ordinary tap water and a brand of bottled water from an internationally recognized producer were used as positive and negative controls.

Methods: Isolation of Organisms: Standard isolation techniques were employed in isolation of organisms. Ten milliliter of each sample was aseptically added to 10 mL of bacteriological peptone water and incubated aerobically overnight at 35±2°C to encourage growth. The broths were then streaked onto Plate Count Agar using the standard calibrated loop for quantification and isolation. All pure isolated colonies were sub-cultured onto Blood agar plates (For growth of heterotrophic bacteria) and MacConkey agar (for coliforms) for 24 h at 35±2°C for colony isolation and morphological identification.

Identification of organisms: Pure isolated colonies were Gram differentiated and then biochemically identified using Indole, Catalase, Citrate, Oxidase, Coagulase and Urease tests (Harrigan and MeCance, 1993).

Antibiotic susceptibility testing: Antibiotic susceptibility were determined by the agar diffusion technique on Mueller-Hinton agar (Kirby-Bauer NCCLS modified disc diffusion technique) using 8 antibiotic discs (Biotec Lab. United Kingdom) corresponding to the drugs most commonly used in the treatment of human and animal infections caused by bacteria; Ampicillin (AMP) (10 μg), Tetracycline (TET) (10 μg), Gentamycin (GEN) (10 μg), Cotrimoxazole (COT) (25 μg), Cefuroxime (CRX) (30 μg), Penicillin (PEN) (15 μg), Flucloxacillin (FLX) (5 μg) and Erythromycin (ERY) (5 μg).

Statistical analysis: Data obtained in the study were analyzed descriptively using Statview from SAS Version 5.0.

RESULTS

The mean microbial counts on Plate Count Agar (PCA) of sampled sachet water brands ranged from 2.8×10^{3}-5.9×10^{5} cfu mL^{-1}. Results of 1st, 2nd and 3rd sampling showed at least one bacterial isolate at each sampling period. An average of three different bacteria spp. was isolated for each sample over the study period. Tap water (positive control) showed a similar growth trend over the sampling period. Bottled water (negative control) had no bacteria growth over the period of sampling (Table 1). A total of 14 different bacteria were isolated from the samples over the sampling period. *S. aureus* was the highest isolate 10 (22.2%) as well as contaminates the most brands of water (8), followed by *E. faecalis* 9 (20%) in 7 brands, *E. coli* 6 (13.3%) in 5 brands, *B. cereus* and *L. monocytogenes* isolated 4 (8.9%) each and in 4 and 3 brands of water respectively. Other isolates such as CN-*Staphylococcus* 3(6.7%) in 3 brands, *M. catarrhalis, Enterobacter* sp., *Serratia marcescens, Shigella* sp. and *Citrobacter* sp. were isolated once (2.2%) in one brand of water each (Table 2). Isolates of *L. monocytogenes, Enterobacter* sp. and *Shigella* sp. showed the highest

Table 1: Bacteria isolates from the different sampling periods

Samples	Isolates in 1st sampling	Isolates in 2nd sampling	Isolates in 3rd sampling	No. of different isolates
1	*E. coli, CN-Staphylococcus*	*E. coli*	*S. aureus, E. faecalis*	4
2	*S. aureus, E. faecalis*	*CN-Staphylococcus*	*E. coli*	4
3	*E. coli, M. catarrhalis,*	*S. marcescens*	*Enterobacter* sp.	3
4	*K. aerogenes, S. aureus,*	*Citrobacter* sp.	*E. faecalis*	4
5	*S. aureus, E. faecalis*	*E. coli, E. faecalis*	*Enterobacter* sp.	4
6	*E. faecalis, K. aerogenes*	*K. aerogenes*	*E. faecalis*	2
7	*L. monocytogenes, E. faecalis*	*B. cereus*	*S. aureus*	4
8	*L. monocytogenes, B. cereus*	*S. aureus*	*L. monocytogenes*	3
9	*S. aureus, E. faecalis*	*Shigella* sp.	*E. coli*	4
10	*B. cereus, CN-Staphylococcus*	*L. monocytogenes*	*B. cereus*	3
11	*B. cereus, S. aureus*	*S. aureus*	*S. aureus*	2
Tap	*L. monocytogenes,*	*Proteus* sp.	*B. cereus*	4
Bottled	No Bacteria Growth (NBG)	NBG	NBG	0

CN-Staphylococcus = Coagulase negative staphylococcus

Table 2: Frequency of bacterial isolates and the number of brands contaminated

Bacteria isolates	Frequency	Percentage	No. of brands contaminated
E. coli	6	13.3	5
E. faecalis	9	20.0	7
S. aureus	10	22.2	8
B. cereus	4	8.9	4
L. monocytogenes	4	8.9	3
CN- Staphylococcus	3	6.7	3
Enterobacter sp.	2	4.4	2
M. catarrhalis	1	2.2	1
Citrobacter sp.	1	2.2	1
S. marcescens	1	2.2	1
Shigella sp.	1	2.2	1

Table 3: Bacteria isolates and their percentage resistance and pattern

Isolates	No. of brands	Resistance patterns[a]	% Resistance[b]
E. coli	5	AMP, FLX, ERY, PEN	50.0
E. faecalis	7	AMP, FLX, ERY, PEN, CRX	62.5
S. aureus	8	AMP, FLX, ERY, PEN, CRX, COT	75.0
B. cereus	4	AMP, FLX, ERY, PEN, CRX, COT	75.0
L. monocytogenes	3	AMP, FLX, ERY, PEN, CRX, COT, TET	87.5
CN- Staphylococcus	3	AMP, FLX, ERY, PEN, CRX, COT	75.0
K. aerogenes	2	AMP, FLX, ERY, PEN, CRX	62.5
Enterobacter sp.	1	AMP, FLX.ERY, PEN, CRX, COT, TET	87.5
M. catarrhalis	1	AMP, FLX, PEN, CRX, COT	62.5
Citrobacter sp.	1	AMP, FLX, ERY, PEN, CRX	62.5
S. marcescens	1	AMP, FLX, ERY, PEN, CRX	62.5
Shigella sp.	1	AMP, FLX, ERY, PEN, CRX, COT, TET	87.5

[a]Resistance pattern constructed from the antibiogram; antibiotic codes as defined under methodology. [b]Percentage Resistance obtained from the antibiogram

Table 4: Number and percentage resistant of bacteria to antibiotics

Antibiotic (µg/disk)	No. of bacteria resistant to antibiotic	% Resistance to antibiotic
Ampicillin (10)	14	100.0
Flucloxacillin (5)	14	100.0
Penicillin (15)	14	100.0
Erythromycin (5)	13	92.9
Cefuroxime (30)	13	92.9
Co-trimoxazole (25)	8	57.1
Tetracycline (10)	3	21.4
Gentamycin (10)	0	0.0

percentage resistance to the antibiotics (87.5%). *E. coli* showed the least resistance of (50%) (Table 3). All the isolates were resistant to Ampicillin, Flucloxacillin and Penicillin (100% resistance) but were all susceptible to Gentamycin (0% resistance) (Table 4).

DISCUSSION

The enumerated heterotrophic bacteria counts conform to a study undertaken by Obiri *et al.* (2003) that isolated similar levels of bacteria in sachet water sold in the streets of Kumasi in Ghana. The presence of faecal coliforms and *E. coli* in the sachet water indicates contamination of this water considered by most people as cleaner water compared with tap water and confirms works by Obiri *et al.* (2003), Dodoo *et al.* (2006) and Oyedeji *et al.* (2010). The faecal contamination of the sachet waters may be due to the fact that the water may have been prepared from shallow and contaminated boreholes which were not properly purified or even filtered at all. High demand for packaged water for various occasions has led to springing up of small scale entrepreneurs who engage in production of packaged waters without due regard to hygienic practices in the production processes. The implication of this is lack of guarantee that the products will meet set standards for drinking water quality. WHO (1996) guidelines values for bacteriological quality for all water intended for drinking stated that *E. coli* or total coliform bacteria must not be detectable in any 100 mL samples. Therefore by World Health Organization standards, five of the sachet water brands were not safe for human consumption. *S. aureus* isolated from the water sample may have entered the water during packaging or handling since the organisms are normal

flora of the human skin (Hunter, 1993). Inadequate sanitation and unhygienic practices account for the major source of microbial contamination of any potable water (Sahota, 2005). This results in microbial contamination of the emerging product. The presence of this organism in drinking water is of public health importance because it is usually responsible for Staphylococcal food poisoning (Hobbs and Robert, 1993; Frazier and Westhoof, 1995). Isolation of pathogenic bacteria such as *Proteus* sp., *Shigella* sp. and *Serratia* sp., *E. faecalis, B. cereus, M. catarrhalis* confirms works by Olaoye and Onilude (2009).

The result of the antibiotic susceptibility testing showed various percentages of antibiotic resistance among the bacterial isolates from sachet water samples. The bacterial isolates showed 100% resistance to Ampicillin, Penicillin and Flucloxacillin which confirms research by Nwachukwu and Otokunefor (2003) in which sixty three Gram negative bacteria isolated from rural untreated drinking stream water supply demonstrated high resistance to Ampicillin. Earlier research by McKeon *et al.* (1995) observed that 87% of coliforms in ground waters supplies were resistant to at least one antibiotic with resistance most commonly directed towards Novobiocin, Cephalothin and Ampicillin. Most of the bacteria isolated were resistant to commonly useful antibiotics such as Ampicillin (100%), Penicillin (100%), Co-trimoxazole (57.1%), Cefuroxime (92.9%), Erythromycin (92.9%) and thus represent a public health concern (Khan and Malik, 2001). Gentamycin, however showed 100% sensitivity conforming to a study by Oyetayo *et al.* (2007), who found similar results in *E. coli* isolated from drinking wells in Nigeria. Bhowmick *et al.* (2006) also observed that bacteria isolated from whey show complete sensitivity to Gentamycin and to some extent Tetracycline but was completely resistant to Ampicillin. The presence of antibiotics resistant bacteria in sachet water is of health significance because of the danger in promoting multiple antibiotic resistant organisms in humans through possible colonization of the gastrointestinal tract and conjugal transfer of antibiotic resistance to the normal flora leading to more multiple antibiotic resistance organisms (McKeon *et al.*, 1995). The prevalence of drug resistant organisms poses a great challenge to clinicians and the consumption of water containing these antibiotic resistant organisms may prolong the treatment of water-borne diseases. This implies that treatment of water-borne diseases with these antibiotics may be inappropriate and will require new and mostly expensive antibiotics. This study therefore exposes the presence of antibiotic resistant bacteria in sachet drinking water with its public health implications and recommends that stringent measures be taken to prevent their occurrence.

ACKNOWLEDGMENT

The authors wish to express their profound gratitude to Andrew Kojo Yarquah, Principal Technician of the Department of Laboratory Technology, UCC, Mr. Daniel Edem Azumah of the Central Regional Hospital's Laboratory, Cape Coast and Mr. Agyemang of the Central Laboratory Services of the Korle-Bu Teaching Hospital, Accra.

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